Hindawi Publishing Corporation
Journal of Biomedicine and Biotechnology
Volume 2009, Article ID 617509, 8 pages
doi:10.1155/2009/617509

Research Article

Genome-Wide Transcriptional Profiling of the Response of *Staphylococcus aureus* to Cryptotanshinone

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Received 1 December 2008; Revised 21 May 2009; Accepted 28 June 2009

Recommended by Daniele Daffonchio

Staphylococcus aureus (*S. aureus*) strains with multiple antibiotic resistances are increasingly widespread, and new agents are required for the treatment of *S. aureus*. Cryptotanshinone (CT), a major tanshinone of medicinal plant *Salvia miltiorrhiza* Bunge, demonstrated effective in vitro antibacterial activity against all 21 *S. aureus* strains tested in this experiment. Affymetrix GeneChips were utilized to determine the global transcriptional response of *S. aureus* ATCC 25923 to treatment with subinhibitory concentrations of CT. Transcriptome profiling indicated that the antibacterial action of CT may be associated with its action as active oxygen radical generator; *S. aureus* undergoes an oxygen-limiting state upon exposure to CT.

1. Introduction

Staphylococcus aureus (*S. aureus*) is one of the most important pathogens in both hospitals and the community and can cause numerous syndromes in humans, such as endocarditis, osteomyelitis, and septicemia [1, 2]. Greater than 60% of *S. aureus* isolates are now resistant to methicillin (oxacillin), and some strains have developed resistance to more than 20 different antimicrobial agents [3]; new agents are therefore needed for the treatment of *S. aureus*.

For over 100 years, chemical compounds isolated from medicinal plants have served as the models for many clinically proven drugs and are now being reassessed as antimicrobial agents [4]. Cryptotanshinone (CT) is a major tanshinone of Dan Shen, the root of *Salvia miltiorrhiza* Bunge (Labiatae) [5]. CT exhibits antimicrobial activity against a broad range of Gram-positive, including *S. aureus*, and Gram-negative bacteria as well as other microorganisms [6, 7].

Although CT exhibited fairly high levels of activity against *S. aureus*, there have been no reports related to the inhibitory mechanisms of CT against *S. aureus*. Transcriptional profilings generated with Affymetrix GeneChips have been used to identify genes of *S. aureus* that were induced in response to the antibiotics vancomycin, oxacillin, d-cycloserine, or bacitracin [8, 9]. Transcriptional profiles of microorganisms treated with an inhibitor could provide valuable information for both pathway characterization, and for determination of the mechanism of inhibition [10, 11].

In this report, *to investigate* the antimicrobial activity and the possible action mechanisms of CT against *S. aureus*, we tested the minimum inhibitory concentrations (MICs) of CT against 21 *S. aureus* strains, as well as utilizing Affymetrix GeneChip analysis to identify differentially expressed genes for *S. aureus* treated with subinhibitory concentrations of CT.

2. Materials and Methods

2.1. Bacterial Strains and Materials. The *S. aureus* strains used in this study were comprised of 20 clinical isolates from the First Hospital of Jilin University, which have different antibiograms against 12 antimicrobial agents (Table 1), and the standard strain ATCC 25923 obtained

from China Medical Culture Collection Center (CMCC). Mueller-Hinton broth II (MHB II) and Mueller-Hinton agar (MHA) were purchased from BD (Biosciences, Inc., Sparks, Md). CT was purchased from the China Medical Culture Collection Centre (CMCC), and stock solutions of varying concentrations were dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich).

2.2. Antibiotic Susceptibility Testing. The minimum inhibition concentrations (MICs) of CT against the 21 *S. aureus* strains were determined in triplicate by broth microdilution or broth macrodilution using twofold serial dilutions in MHB II, according to CLSI/NCCLS M100-S15. The MICs were defined as the lowest concentration at which no visible growth was observed. The minimum concentration of CT that inhibited 90% of the isolates tested was defined as the MIC_{90}.

2.3. Synergistic Study. A standard checkerboard assay was performed to assess the interaction of combination (CT and TMP/SXT) against *S. aureus* strain ATCC 25923 by a well-established method [12]. The fractional inhibitory concentration (FIC) index was calculated by a previously described method [12]. An FIC index (FICI) between two compounds less than or equal to 0.5 is considered synergism, an FIC index between 0.5 and 2 is considered indifference, and a FICI equal to or more than 2 is considered antagonism.

2.4. Growth Curves. *S. aureus* strain ATCC 25923 was grown to an optical density of 0.3 at 600 nm in MHB II, and 100 mL aliquots were then distributed into five 500 mL Erlenmeyer flasks. CT (dissolved in DMSO) was added to four of the cultures to obtain final concentrations of 1/4 × MIC (1 μg·mL), 1/2 × MIC (2 μg·mL), MIC (4 μg·mL), and 2 × MIC (8 μg·mL), respectively. The final DMSO concentration used was 1% (vol/vol), and the control culture was supplemented with 1% DMSO. The cultures were incubated, and cell growth was spectrophotometrically monitored as the optical density at 600 nm, which was recorded at certain time intervals.

2.5. Treatment with CT. *S. aureus* strain ATCC 25923 was grown overnight at 200 rpm in a rotary shaker at 37°C in 10 mL of MHB II. Six 250-mL Erlenmeyer flasks, each containing 100 mL of MHB II, were inoculated with an overnight culture to an initial OD_{600} of 0.05. The bacteria were then grown at 37°C at 200 rpm to an OD_{600} of 0.3. Subsequently, 500 μL of a 12 800 μg·mL CT stock solution, prepared in dimethyl sulfoxide (DMSO), was added to three of the cultures (experimental cultures), yielding a final concentration of 1/2 × MIC (2 μg·mL). Hence, the final concentration of solvent in each CT treatment was 1% (vol/vol) DMSO, which did not alter the pH of the medium. The other three cultures lacking CT and supplemented with 1% (vol/vol) DMSO were used as the control. All bacterial suspensions (both CT treatment or control condition) were further incubated for 30 minutes at 37°C for RNA isolation.

2.6. RNA Isolation and cDNA Labeling. Bacterial cells were treated with RNA Protect bacterial reagent (QIAGEN, Inc., Valencia, Calif) to minimize RNA degradation immediately before harvesting. Cells were collected by centrifugation and stored at −80°C. RNA isolation and cDNA labeling were performed as previously described [13]. Three independent RNA preparations and cDNA labelings were performed on different days.

2.7. GeneChip Hybridization and Analysis. The GeneChip *S. aureus* genome array (antisense) was provided by CapitalBio Corporation (http://www.capitalbio.com/index.asp, Beijing, China), a service provider authorized by Affymetrix Inc. (Santa Clara, CA). This GeneChip includes N315, Mu50, NCTC 8325, and COL. The array contains probe sets to over 3 300 *S. aureus* ORFs and over 4 800 intergenic regions. GeneChip hybridization, washing, staining, and scanning were performed as previously described [13].

The images were processed with Microarray Analysis Suite 5.0 (Affymetrix). The raw data from the array scans were normalized by median-centering genes for each array, followed by log transformation. Expressed genes were identified using Affymetrix GeneChip Operating Software (GCOS, Ver.1.0), which utilizes statistical criteria to generate a "present" or "absent" call for genes represented by each probe set on the array. Additionally, genes with "absent" scores were filtered out of the dataset, and the remaining genes were analyzed. To identify genes that are differentially expressed in CT-treated samples compared to controls, the Significance Analysis of Microarrays (SAM) software (http://www-stat.stanford.edu/~tibs/SAM/index.html) was used. To select the differentially expressed genes, we used threshold values of ≥1.5- and ≤−1.5-fold change between three RH treatment samples and three control samples; the FDR significance level was <5%.

2.8. Quantitative Real-Time RT-PCR. Quantitative real-time reverse transcription (RT)-PCR was used to verify the microarray results. Aliquots of the RNA preparations from CT-treated and control samples used in the microarray experiments were also used for quantitative real-time RT-PCR follow-up studies. The cDNA was subjected to real-time PCR using the primer pairs listed in Table 2. Quantitative real-time PCR was performed in triplicate using the 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA), as previously described [13].

3. Results and Discussion

3.1. MICs of CT Against S. aureus Strains and Influence of CT on Growth Curves. In this experiment, the MICs of CT against 21 *S. aureus* strains ranged from 4 to 64 μg·mL (Table 1), and the MIC_{90} was 16 μg·mL. The MIC value of *S. aureus* strain ATCC 25923 versus CT was 4 μg·mL. This result demonstrated that CT is a potentially effective antimicrobial against *S. aureus*.

We performed growth curve of *S. aureus* ATCC 25923 with CT (1, 2, 4, 8, and 16 μg·mL) added at the time

Table 1: Antibiograms of 21 *Staphylococcus aureus* strains used in this study.

Strains	Source	Date of isolation (mo/yr)	Antibiotic resistances	V MIC (μg·mL)	CIP MIC (μg·mL)	OX MIC (μg·mL)	CT MIC (μg·mL)
SA003	Blood	2/2003	P	1	0.12	0.12	4
SA006	Skin	2/2003	M, P, CI, CM, E	1	64	128	8
SA009	Skin abscess	3/2003	M, CI, CM, E	0.5	128	256	16
SA017	Skin abscess	5/2003	P, T	2	0.5	0.5	4
SA018	Broncheal swab	6/2003	M, P, CI, CM, E, G	2	128	512	16
SA025	Sputum	10/2003	—	0.25	0.25	0.12	4
SA039	Broncheal swab	2/2004	M, P, CI, CM, E, G, RI, T, TMP/SXT	1	512	512	64
SA059	Broncheal swab	6/2004	M, P, CI, T, TMX/SXT	2	128	512	8
SA079	Sputum	9/2004	M, P, CI, CM, E, G, TMP/SXT	4	128	256	32
SA092	Abscess	10/2004	M, P, E	1	16	128	16
SA106	Wound isolate	1/2005	M, P, CI, CM, E, G, T, TMP/SXT	0.5	256	512	16
SA118	Blood	1/2005	M, P, E, G	1	64	256	16
SA121	Wound isolate	3/2005	M, P, CM, E, G	1	128	256	16
SA142	Abscess	5/2005	P, T, E	1	1	0.5	8
SA146	Skin abscess	5/2005	M, P, CM, E, T	2	64	128	16
SA165	Urine	6/2006	P, CI, CM, E, G, RI, T	2	256	512	16
SA173	Broncheal swab	8/2005	M, P, T	0.25	32	128	8
SA179	Blood	9/2005	M, CI, CM, E	1	128	512	16
SA192	Urine	10/2005	M, P, CI, CM, E, G	1	64	512	16
SA203	Wound isolate	12/2005	M, P, CM, T	2	64	512	16
ATCC25923	CMCC	—	—	1	0.25	0.25	4

Abbreviations: M: methicillin; P: penicillin; CI: clindamycin; CM: chloramphenicol; E: erythromycin; G: gentamicin; RI: rifampin; T: tetracycline; TMP/SXT: trimethoprimsulfamethoxazole; V: vancomycin; CIP: Ciprofloxacin; OX: Oxacillin.

point of OD600 = 0.3. Equivalent DMSO was added to the experimental cultures and control cultures. Bacterial cells samples were collected at certain time intervals in this experiment with a time course of 1440 minutes, and the growth curves of CT-treated and untreated strain were measured spectrophotometrically. With 1 and 2 μg·mL of CT and controls, we found the optical density increased steadily, while with 4 μg·mL, 8 μg·mL, and 16 μg·mL of CT, we did not observe increase in the optical density (Figure 1). This result showed that CT displays bacteriostatic action against *S. aureus*, which is consistent with previous reports [15, 16].

3.2. Gene Transcription Responses to CT Exposure. GeneChip analysis revealed that a substantial number of genes (185) were differentially regulated in response to CT. Of these, 100 genes exhibited a significant increase in transcript abundance, and 85 exhibited a significant decrease in transcript abundance. The microarray-related data were submitted to Gene Expression Omnibus (GEO) under the accession number: **GSE13203**. The distribution of CT-responsive genes and their biological roles are shown in Figure 2. A complete list of all genes differentially expressed by CT can be found in the supplemental material (See supplementary Table S1 in supplementary material available online at doi: 10.1155/2009/617509). Herein, our interest mainly focused on specific genes that may allow the organism to survive in the presence of CT.

3.3. Genes of the Sortase Enzyme and Iron-Regulated Surface Determinants (Isd) are Inhibited in Response to CT. In the present study, the sortase-encoding gene *srtB* (SA0982) was repressed 3-fold, and some surface protein-encoding genes of *isdABDEFG* were also significantly inhibited in response to CT. The Isd cluster of *S. aureus* is composed of 10 genes (*isdABCDEFGHI* and *srtB*). Pathogens, such as *S. aureus*, require iron for survival and have evolved specialized proteins, such as Isd, to scavenge heme from their host [17]. Therefore, the staphylococcal Isd system can be viewed as a pathogenic strategy for scavenging heme iron during infection by tapping into the iron rich source of hemoproteins

Table 2: Primers used in real-time RT-PCR with SYBR green probes.

Primer	N315 ORF[a]	Sequence
16S rRNAfor[b]	SArRNA01	CGTGCTACAATGGACAATACAAA
16S rRNArev[b]	SArRNA01	ATCTACGATTACTAGCGATTCCA
ribAfor	SA1587	GCTTACATTCTGCGTGCCTTAC
ribArev	SA1587	ACAATCCTATGCCACGACCT
isdAfor	SA0977	GCAACAGCGAAATCTGAAAG
isdArev	SA0977	AAGGCAACTGTGCTAATAAAG
modAfor	SA2074	TTAACTATGGTGGATCAGGG
modArev	SA2074	CATGCGCTTTATTCTTGTCT
seofor	SA1648	AGTCAAGTGTAGACCCTATT
seorev	SA1648	AGATATTCCATCTAACCAAT
SA1831for	SA1831	CGTGGTTTATCAACTTGAGT
SA1831rev	SA1831	TATTCTTTAATTCTTGTGCG
srtBfor	SA0982	TAAATAAAGACATTGTTGGATGG
srtBrev	SA0982	TACGTCGATGTTCTCGCTCA
glpTfor	SA0325	CGACTTTGCTACAAGCGATAA
glpTrev	SA0325	CGCCCAATCAAGTACACCA
oppFfor	SA0198	TCAGAAGGCTTTATTTGGT
oppFrev	SA0198	GAATGATTAGTGGTCGTTTA
SA2135for	SA2135	AACTTAAAGCCTCAACATAG
SA2135rev	SA2135	TAACTTCCAACTGCCATAC
msmXfor	SA0206	CATTTGGGCTAAAGCTACG
msmXrev	SA0206	GACGCTGTCCACCAGATAA
opp-1Ffor	SA2251	TCATCATTACACCCATTTC
opp-1Frev	SA2251	GCCTTAGATAGACCGACTT
dpsfor	SA1941	TTAGCGGTAGGAGGAAAC
dpsrev	SA1941	ATCATCGCCAGCATTACC
SA0302for	SA0302	GAATGGAAAAACAGGAAAAC
SA0302rev	SA0302	GCAAACACATAGCCAATAAG

[a] ORF, open reading frame.
[b] refer to references [14].

[18]. Our results suggest that CT significantly affected the staphylococcal Isd system. In our study, the transcriptional level of *srtCHI* was not differentially modulated by a factor of ≥ 2.0 or ≤ -2.0 upon exposure to CT.

3.4. Anaerobic Respiration and Fermentation, and Oxidative Stress Resistance Genes Regulated by CT. The expression of some genes involved in anaerobic respiration and fermentation was induced by CT during this study, including *fdaB*, *pflB*, *pflA*, *nirB*, *nirD*, *narG*, *narH*, and *narI*.

The *fdaB* gene encodes fructose-bisphosphate aldolase. The transcription of the *fdaB* gene, which is involved in glycolysis, was upregulated, indicating enhanced glycolytic activity by CT. Similar upregulation results were obtained for *fdaB* under anaerobic conditions [19]. The *pflB* and*pflA* genes encode enzymes of the fermentation pathways, which are involved in acetate and ethanol formation [19]. Prior studies demonstrated that the *pflA*/*pflB* pair is involved in energy metabolism when *E. coli* grows under oxygen-limiting conditions [20]. NarG (respiratory nitrate reductase, alpha subunit), NarH (respiratory nitrate reductase, beta subunit), and NarI (respiratory nitrate reductase, gamma subunit) have been shown to be involved in nitrate respiration in *E. coli* [21]. NirB (nitrite reductase, large subunit) and NirD (nitrite reductase, small subunit) are essential and sufficient for NADH-dependent nitrite reduction [22]. Furthermore, *S. aureus* is able to use nitrate or nitrite as alternative electron acceptors [19, 23]. Transcripts of the *nar* and *nir* operon were found to be present at elevated levels under anaerobic conditions [19].

Additionally, CT also induced the *arcABCD* genes, which are associated with energy metabolism. The *S. aureus* arginine deiminase (ADI) metabolic pathway genes are organized in an operon *arcABDC* [24]. The proteins encoded by *arcABCD* are responsible for the ADI pathway, which enables arginine-dependent anaerobic growth [25]. Oxygen deficiency promotes the autophosphorylation of ArcB that activates phosphorylation of ArcA and results in regulation of numerous operons that provide control of carbon catabolism and cellular redox status [26]. Consequently, our results described herein imply that CT may induce anaerobic respiration and fermentation in *S. aureus*.

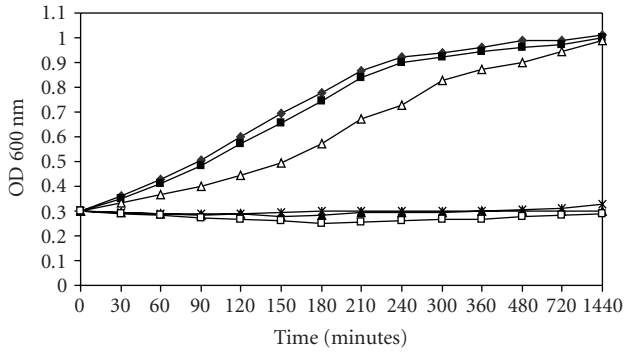

Figure 1: Growth curve for *S. aureus* strain ATCC 25923 in the presence or absence of CT. ■, *S. aureus* ATCC 25923 plus 1 μg·mL CT; Δ, *S. aureus* ATCC 25923 plus 2 μg·mL CT; ∗, *S. aureus* ATCC 25923 plus 4 μg·mL CT; ▲, *S. aureus* ATCC 25923 plus 8 μg·mL CT; □, *S. aureus* ATCC 25923 plus 16 μg·mL CT; ♦, untreated *S. aureus* ATCC 25923.

Expression of some genes involved in oxidative stress resistance was affected by CT, including *ahpC*, *ahpF*, *katA*, and *sodM*. The *sodM* gene encodes superoxide dismutase, and *katA* encodes the catalase enzyme. *KatA* is the only major catalase in *S. aureus*, accumulates extracellularly, detoxifies H_2O_2, and was proposed to be a major virulence determinant in *S. aureus* [27, 28]. Our results showed upregulation of the *katA* gene and downregulation of the *sodM* gene, thus connecting CT to oxidative-stress response. Surprisingly, a similar transcriptional response also occurs when cells are subjected to 1 mM peracetic acid [29]. The *ahpC* gene encodes alkyl hydroperoxide reductase subunit C, and *ahpF* encodes alkyl hydroperoxide reductase subunit F. Our results also demonstrated an upregulation of the *ahpC* and *ahpF* genes. Alkyl hydroperoxide reductase subunit C (AhpC) protects cells against OONO2, which is generated within neutrophils and macrophages [30].

Recent report suggested that superoxide radical formation might be the cause of antibacterial activity of CT [7]. As mentioned above, our results showed clearly that genes *fdaB*, *pflB*, *pflA*, *nirB*, *nirD*, *narG*, *narH*, and *narI* involved in anaerobic respiration and fermentation were upregulated, and genes *ahpC*, *ahpF*, and *katA* involved in oxidative stress resistance were upregulated by CT. Chang et al. indicated that hydrogen peroxide, a reactive oxygen species, led to genes *pflBA* and *arcBC* increases in transcription levels; they suggested that *S. aureus* might undergo an oxygen-limiting state in response to hydrogen peroxide-driven oxidative stress [31]. Further, previous result showed that in *E. coli pfl* is significantly induced by shifting the culture condition from an aerobic to a microaerobic state [32]. Moreover, transcriptome and proteome analysis of *Bacillus subtilis* gene expression in response to superoxide and peroxide stress showed that genes *katA* and *ahpCF* were significantly induced [33]. Thus, our microarray result is consistent with previous observations which revealed that CT might act as superoxide radicals generator; Lee et al. proposed that this phenomenon benefited *S. aureus* by preventing further cytotoxicity arising from reactive oxygen species produced during oxygen respiration [7]. Lactoquinomycin A (LQMA), an antibiotic having a quinone moiety like CT, also generated superoxide radicals during reduction of the quinone moiety by quinone reductase and resulted in other active oxygens [7].

3.5. Antibiotics Resistance Genes Affected by CT. Genechip analysis showed that several antibiotic resistance genes were differentially regulated by CT exposure, including *dfrA*, *drp35*, *cdsA*, and *pgsA*. Among these, the transcription of *dfrA* was upregulated, whereas the transcription of *drp35*, *pgsA* (−2.0-fold), and *cdsA* was significantly downregulated by CT exposure. The *dfrA* gene encodes dihydrofolate reductase, which is responsible for trimethoprim resistance (Tp^r) [34]. In present study, we found that CT has high MIC values in trimethoprimsulfamethoxazole (TMP/SXT) susceptible and resistant strains. In order to test the interaction between CT and TMP/SXT, we conducted additional experiment to assay the vitro antimicrobial activity of CT against *S. aureus* strain ATCC 25923 in combination with TMP/SXT using checkerboard microdilution method. The result showed that there is an antagonism in combination of CT and TMP/SXT against *S. aureus* 25923, with FICI of 4. It is indicated that the increased expression of gene *dfrA* induced by CT might increase the resistance of the *S. aureus* to TMP/SXT. This result suggested that it is necessary to avoid simultanous use of CT and TMP/SXT. The *drp35* gene encodes Drp35, which possesses calcium-dependent lactonase activity and is a cytoplasmic protein induced by cell wall-affecting antibiotics or detergents [35]. Antibiotic susceptibility experiments using a *drp35*-defective strain and an overexpressing strain of *S. aureus* revealed that Drp35 is correlated with bacitracin resistance [36]. Two essential genes, *cdsA* and *pgsA*, encoding phosphatidate cytidylyltransferase and phosphatidylglycerophosphate synthase, respectively, are required for synthesis of phosphatidylglycerol phosphate, which might be converted into phosphoglycerol by a nonspecific phosphatase [37]. Conditional lethal alleles of genes involved in phospholipid biosynthesis in *S. aureus*, including *pgsA* and *cdsA*, have also been found to confer hypersusceptibility to macrolides [38].

3.6. Other Genes Differentially Regulated by CT. We found that 2 carotenoid pigment-associated genes, *crtM* and *crtN*, of *S. aureus* were upregulated in response to CT. Carotenoids are natural pigments with important biological activities [39–41]. The species description of *S. aureus* reflects the colony color (L. *aureus*: golden or orange) and distinguishes this species from *Staphylococcus epidermidis* (formerly *Staphylococcus albus*) [42]. Additionally, CT exposure also induced the *ald* gene that encodes aldehyde dehydrogenase, which is associated with biosynthesis of C30 carotenoids in *S. aureus*. The *ald* gene was located between *crtN* and *crtNb* in the same gene cluster [43].

Moreover, some putative transporter genes were differentially regulated by CT exposure. In our study, a large number of putative transporter genes (39) were differentially regulated upon exposure to CT; 12 of these genes were

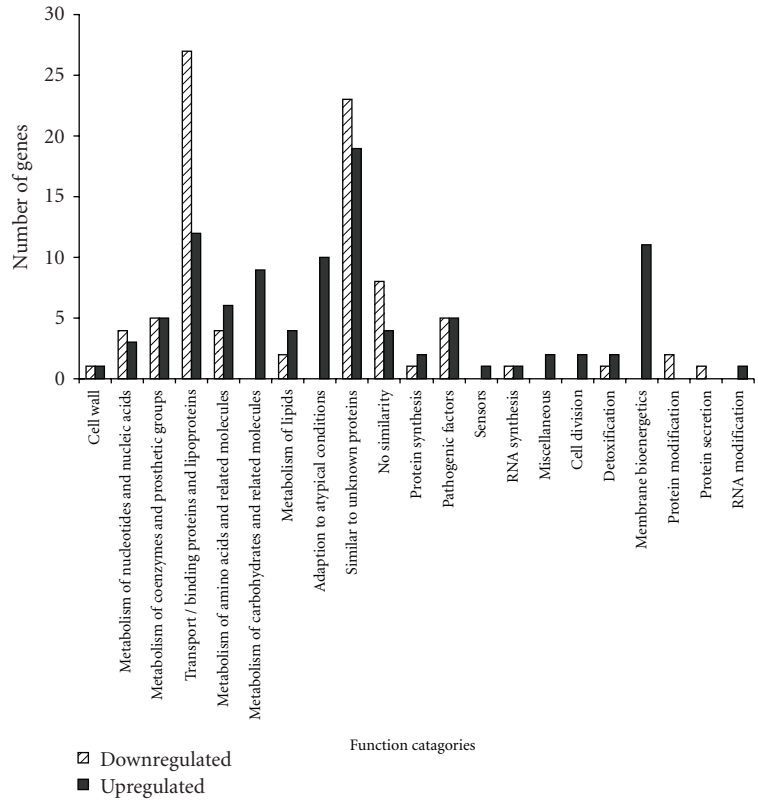

Figure 2: CT-responsive genes grouped by functional classification. The differentially regulated genes were divided into 33 functional categories. The number of genes upregulated and downregulated for each functional group was represented.

Table 3: Real-time RT-PCR analysis of gene expression.

N315 ORF	N315 gene	N315 description	Fold change ± SD[(a)]	
			RT-PCR	Microarray
SA1587	*ribA*	Riboflavin biosynthesis protein	−3.8 ± 1.9	−2.5 ± 1.3
SA0977	*isdA*	Cell surface protein	−4.3 ± 2.3	−3.2 ± 1.0
SA2074	*modA*	Probable molybdate-binding protein	−2.8 ± 0.9	−2.5 ± 0.8
SA1648	*seo*	Enterotoxin SeO [Pathogenicity island SaPIn3]	−3.8 ± 1.2	−2.5 ± 0.6
SA1831		Hypothetical protein [Pathogenicity island SaPIn1]	−19.4 ± 6.1	−6.1 ± 1.6
SA0982	*srtB*	NPQTN specific sortase B	−3.2 ± 0.8	−3.0 ± 1.1
SA0325	*glpT*	Glycerol-3-phosphate transporter	−2.7 ± 0.3	−2.8 ± 0.4
SA0198	*oppF*	Oligopeptide transport ATP-binding protein	−2.8 ± 0.5	−2.5 ± 0.8
SA2135		Hypothetical protein, similar to sodium/glutamate symporter	−2.8 ± 0.8	−2.1 ± 0.8
SA0206	*msmX*	Multiple sugar-binding transport ATP-binding protein	−3.4 ± 1.0	−2.5 ± 0.8
SA2251	*opp-1F*	Oligopeptide transporter putative ATPase domain	−2.6 ± 0.7	−2.6 ± 0.6
SA1941	*dps*	General stress protein 20U	10.5 ± 2.8	4.9 ± 1.6
SA0302		Probable pyrimidine nucleoside transport protein	18.4 ± 5.4	6.1 ± 1.7

[(a)] + indicates reduction, and − indicates increase. Standard deviation were calculated based on three independent experiments.

upregulated, while 27 were downregulated. These genes included 26 ATP-dependent transporter genes (all involved in the ATP-binding cassette (ABC) superfamily) and 12 secondary transporter genes (3 genes are associated with the major facilitator superfamily (MFS)). In addition, a phosphotransferase system (PTS) gene, *mtlA* (SA1962), was expressed at an increased level. Among the putative transporter genes regulated by CT, the *semB* gene is a multidrug resistance (MDR) transporter, which was expressed at an increased level of 2.8-fold.

3.7. Validation of Microarray Data by Real-Time RT-PCR. Real-time quantitative RT-PCR was conducted to validate microarray data using the same RNA from the original microarray experiment, and 13 genes were selected for this analysis (i.e., *ribA*, *isdA*, *modA*, *seo*, SA1831, *srtB*, *glpT*, *oppF*, SA2135, *msmX*, *opp-1F*, *dps*, and SA0302). In general, there were positive correlations between microarray data and real-time RT-PCR data for all 13 genes (Table 3). However, expression of the genes SA1831 and SA0302 was observed at greater n-fold changes by real-time RT-PCR than with microarray analysis, indicating that real-time RT-PCR analysis may be more sensitive to changes than microarray analysis in transcript levels. For the remaining 11 genes (i.e., *ribA*, *isdA*, *modA*, *seo*, *srtB*, *glpT*, *oppF*, SA2135, *msmX*, *opp-1F*, and *dps*), the levels of gene induction did not differ markedly between microarray data and real-time RT-PCR data.

4. Conclusion

In summary, the presented antibacterial activity and growth curve experiments demonstrated that CT is a bacteriostatic agent against *S. aureus*. Transcriptional profiling revealed that the action mechanism of CT on *S. aureus* is correlated to its action as active oxygen radical generator; *S. aureus* might undergo an oxygen-limiting state upon exposure to CT. To our knowledge, this genome-wide transcriptomic approach revealed the first insights into the response of *S. aureus* to CT challenge. Recognition of CT lays the groundwork for developing new agents owning good activity based on chemical structure modification of the lead compound.

Acknowledgments

The authors are grateful to Professor Liang Zhang (CapitalBio) for Affymetrix microarray work and helpful discussions. This work was supported by the National Nature Science Foundation of China (no. 30871889).

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